# Myopia and Late-Onset Progressive Cone Dystrophy Associate to LVAVA/MVAVA Exon 3 Interchange Haplotypes of Opsin Genes on Chromosome X

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**Purpose.** Rare interchange haplotypes in exon 3 of the *OPN1LW* and *OPN1MW* opsin genes cause X-linked myopia, color vision defect, and cone dysfunction. The severity of the disease varies on a broad scale from nonsyndromic high myopia to blue cone monochromatism. Here, we describe a new genotype–phenotype correlation attributed to rare exon 3 interchange haplotypes simultaneously present in the long- and middle-wavelength sensitive opsin genes (L- and M-opsin genes).

**Methods.** A multigenerational family with X-linked high myopia and cone dystrophy was investigated.

**Results.** Affected male patients had infantile onset myopia with normal visual acuity and color vision until their forties. Visual acuity decreased thereafter, along with the development of severe protan and deutan color vision defects. A mild decrease in electroretinography response of cone photoreceptors was detected in childhood, which further deteriorated in middle-aged patients. Rods were also affected, however, to a lesser extent than cones. Clinical exome sequencing identified the LVAVA and MVAVA toxic haplotypes in the *OPN1LW* and *OPN1MW* opsin genes, respectively.

**Conclusion.** Here, we show that LVAVA haplotype of the *OPN1LW* gene and MVAVA haplotype of the *OPN1MW* gene cause apparently nonsyndromic high myopia in young patients but lead to progressive cone–rod dystrophy with deuteranopia and protanopia in middle-aged patients corresponding to a previously unknown disease course. To the best of our knowledge, this is the first report on the joint effect of these toxic haplotypes in the two opsin genes on chromosome X.

Keywords: X-linked high myopia, cone dystrophy, LVAVA

The long- and middle-wavelength sensitive opsin genes (L- and M-opsin genes; *OPN1LW*, *OPN1MW*, respectively) reside in a head-to-tail tandem array on Xq28 and share 98% nucleotide identity.[1] The normal gene array is composed of a locus control region (LCR), a single L gene followed by one or more M genes with only the first two genes in the array expressed due to their proximity to the upstream cis-regulatory LCR.[2–5] Mutations in the LCR, L- (*OPN1LW*), and M- (*OPN1MW*) opsin genes on Xq28 lead to various X-linked cone-photoreceptor disorders including red-green color vision defects (Online Mendelian Inheritance in Man [OMIM] #303800 and #303900), X-linked cone dysfunction (Bornholm Eye Disease [BED], OMIM #300843), X-linked cone dystrophy, and blue cone monochromatism (BCM; OMIM #303700).[1,4,6–25] Rare exon 3 interchange haplotypes are a recently described group of mutations of the opsin genes on chromosome X, which involve amino acid residues 153, 171, 174, 178, and 180, and their name is the acronym of the one letter code of specific amino acids present at these locations. Normal opsin genes specify the haplotypes LVAIS and MVAIA in the L and M genes, respectively. Toxic opsin variants that have been discovered in human opsin-related diseases so far include LVAVA, LIAVA, LIAVS, LIVVA, MVVVA, and MIAVA.[4,11,26–31] Patients reported so far with an exon 3 interchange haplotype are summarized in the Supplementary Material. Toxic exon 3 interchange haplotypes have been commonly reported in BED.[14,26,29,32–34] BED was originally described in a family from the Danish island of Bornholm.[32] The second family with BED from Minnesota had also a Danish origin.[14] Affected males had infantile onset myopia with astigmatism, decreased visual acuity from childhood, subnormal photopic, and normal scotopic electroretinogram (ERG) parameters with normal macular appearance. The disease showed no progression with age. The first family had a deutan while the second had a protan color vision defect. The disease was accounted for by the LVAVA interchange haplotype in one of the first two genes in the opsin gene array in both

families. This observation was the first to identify LVAVA haplotype as a cause of BED.[26] LVAVA haplotype associated to a similar phenotype with either protan or deutan color vision defect in four individuals from four recently reported families.[28] Rare interchange haplotypes have been shown to cause skipping of exon 3, a frame shift and premature termination of translation. However, in contrast to the LIAVA haplotype, there was a residual low level of correctly spliced opsin mRNA with the LVAVA or MIAVA haplotypes observed indicating some amount of functionally normal opsin proteins.[29,35] Accordingly, color vision defect was explained by the lack of either L- or M-opsin genes in the first two positions of the opsin gene array instead of a direct opsin inactivating effect in patients with the LVAVA haplotype.[26] These observation suggests that the LVAVA haplotype has a dominant effect on the development of myopia, a key element of BED, however, it is transcribed at a sufficient level to provide normal opsin function.[26] The strongest clinical evidence supporting this hypothesis is the report on two Chinese families with nonsyndromic high myopia associating to the LVAVA haplotype.[33,34] However, this represents a strikingly different phenotype than observed in BED patients with the same haplotype. Interestingly, the LVAVA haplotype is unique among interchange mutations in another aspect as well. According to its definition, BED is a nonprogressive disease. However, a spectral-domain optical coherence tomography (SD-OCT) and adaptive optics scanning laser ophthalmoscopy analysis of two patients with an LVAVA-only genotype showed characteristic signs of progressive macular dystrophy including overall retinal thinning, mottling of photoreceptor inner segments, and structural disruption of the inner retina and the outer nuclear layer.[27] This finding indicated that LVAVA haplotype causes progressive degenerative changes that result in damage to neighboring cells in addition to those expressing the mutated opsin. Moreover, these patients gradually developed clinical signs of BED during their elementary school years, representing a somewhat later disease onset than observed in other BED patients.[27] Taken together, our understanding on the genotype–phenotype correlation associating to the LVAVA haplotype has largely developed since the first description of BED as a stationary disease with color vision deficiency. Recent papers highlight that LVAVA expressing cones remain functional in early life and degenerate later.[27,29,33–35] Phenotypic variability observed in patients with the LVAVA haplotype can presumably be accounted for by other genetic modifiers, the mutational status of the opposite opsin gene, and the number of cones expressing the mutant opsin in the retina. In the present paper, we describe a large multigeneration family with X-linked high myopia and late onset cone dysfunction. The disease is caused by the rare interchange haplotypes LVAVA and MVAVA in the L- and M-opsin genes, respectively. This combination has not been reported so far and provides a unique opportunity to investigate the joint effect of these haplotypes. Moreover, the large multigeneration family provides excellent possibility to observe disease phenotype across different generations and gain insight into disease progression. Besides, we followed the proband for 8 years in order to document disease progression in a single individual. Our results shed light on a new phenotype of cone dystrophy associating to the LVAVA/MVAVA rare interchange haplotypes in the L- and M-opsin genes. This is the first report on the combination of LVAVA and MVAVA haplotypes in the L- and M-opsin genes, respectively. Our findings provide robust clinical evidence for late onset progressive cone dystrophy indicating that these haplotypes have little or no effect on color vision and visual acuity in early life but lead to slowly progressive late-onset cone–rod dystrophy characterized by a progressively declining visual acuity along with color vision deficiency in patients older than 40 years.

## Methods

### Patients and Ophthalmology Investigations

In the present study, we have investigated members of a three-generation family with X-linked high-grade myopia. Autorefractometry was performed using a Topcon KR 8100 equipment (Topcon Corp., Tokyo, Japan). Color vision was tested under standardized conditions, with pseudoisochromatic plates (Tafeln und Prüfung des Farbensinnes, 29. Auflage, 2002), the Farnsworth Munsell 100-hue test and the anomaloscope Nagel type II test. Color fundus photographs were taken with a Zeiss FF450+IR fundus camera (Carl Zeiss AG, Jena, Germany) mounted with a ZK-5 color sensor (Allied Vision Technologies GmbH, Stadtroda, Germany) operated with Zeiss Visupack 4.4 software. Optical coherence tomography and confocal-scanning laser fundus autofluorescence (FAF) imaging was performed with Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany). Electroretinography was executed with Ganzfeld Q400 equipment (Roland Consult GmbH, Brandenburg, Germany) using standard International Society for Clinical Electrophysiology of Vision parameters.[36] Measures of photoreceptor dysfunction were defined as: mild (70%–99% of normal amplitude), moderate (30%–69% of normal), severe (1%–29% of normal), or undetectable. The research was performed according to the Declaration of Helsinki and was approved by the local institutional review boards. Written informed consent was obtained from participating family members prior to investigation.

### Isolation of Genomic DNA

Genomic DNA was isolated from EDTA or citrate anticoagulated blood using the QIAamp Blood Mini kit according to the manufacturer's instructions (Qiagen GmbH, Hilden, Germany).

### Comparative Genomic Hybridization Array

The copy number variations (CNVs) of X chromosome were analyzed by comparative genomic hybridization array (array CGH: Roche, NimbleGen, Madison, WI, USA) on genomic DNA from the proband sent to the NimbleGen custom microarray services facility (NimbleGen Systems of Iceland, LLC, Reykjavik, Iceland). The genomic DNA labelled with Cy3 or Cy5 using NimbleGen Dual Color DNA labelling kit and co-hibridizyed to the arrays (Nimble Gen CGH Services: Guide to your CGH data v5p1). The design of the array was based on the human reference genome NCBI36/hg18.

### Sanger Sequencing of RPGR ORF15

The genomic DNA of the index patient was screened for disease-associated sequence alterations in exon ORF15 of *RPGR* gene by sequencing of PCR products. ORF15 is an alternative 3′ terminal exon that contains exon 15 and extends into intron 15.[37] The amplification consisted of 35 cycles. We applied previously published primers.[38] Cycling conditions of PCR reactions were in case of 15.1, 15.2, 15.3, and 15.5 fragments: initial denaturation 95°C for 10 minutes, denaturation 94°C for 30 seconds, annealing 55°C for 30 seconds, and elongation 72°C for 1 minute. The amplification consisted of 40 cycles. The conditions of the 982-bp length 15.4 fragment was: initial denaturation 95°C for 10 minutes, denaturation 94°C for 45 seconds, annealing 60°C for 45 seconds, and elongation 72°C for 1.5 minutes. Polymerase chain reaction amplicons

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were identified with ABI BigDye Terminator cycle sequencing kit version 3.1 (Applied Biosystems, Foster City, CA, USA) on an ABI 310 sequencer (Applied Biosystems). The sequenced profiles were compared with the retinitis pigmentosa GTPase regulator (RPGR) reference genome (NM_001034853) in National Center for Biotechnology database.

### Clinical Exome Sequencing

Clinical exome sequencing was performed on the sample of the proband (V:1) and of the mother of the proband (IV:3). Exome sequencing was carried out by TruSight One Sequencing Panel (Illumina, San Diego, CA, USA) that covers 4813 clinically relevant genes. Library preparation was done according to the manufacturer's instruction and sequencing was performed using Illumina Miseq system (Illumina). At least 20-fold coverage was observed in 92% of the target regions. The reads were mapped against the human reference genome NCBI37/hg19. For bioinformatic analysis NextGene Software version 2.3.4 was used (SoftGenetics, State College, PA, USA). The analysis pipeline was based on X-linked recessive inheritance. The coding regions of X-linked genes and the splicing regions (±5 bp) were analyzed. The silent and noncoding variants were excluded. Ensembl, HGMD, and ExAC databases were used for analysis of the variants.

### Validation and Segregation Testing of OPN1LW, OPN1MW, and CACNA1F Variants

Putative variants detected by clinical exome sequencing in *OPN1LW, OPN1MW* (c.532A>G/p.Ile178Val and c.538T>G/p.Ser180Ala), and *CACNA1F* (c.1843G>T/p.Ala615Ser) genes were confirmed by Sanger sequencing using sequence specific primer pairs: OPN1LW/OPN1MW F 5′-TAAGCAGGACAGTGG GAAGC-3′; OPN1LW/OPN1MW R 5′-GGCCCAGAGAAAGGAA GTG-3′, CACNA1F-F: GCCTCTCTCCCCACAGAGTA and CAC NA1F-R: TGCTCAATGAATGGTGAAGC. Cycling conditions of PCR reaction were: initial denaturation 95°C for 10 minutes, denaturation 94°C for 30 seconds, annealing 55°C for 30 seconds, and elongation 72°C for 1 minute. Polymerase chain reaction amplicons were sequenced on an ABI310 sequencer (Applied Biosystems). These analyses were performed in all available family members (IV:3, IV:5, IV:7, V:1, V:3, VI:6).

## Results

### Clinical Findings

We have investigated a three-generation family with X-linked high myopia. Three of the precedent generations were also included in the family tree (Fig. 1). Living family members unanimously confirmed that male family members of the second generation suffered from serious visual disability, however its cause remained undisclosed. (Subject IV:9 refused to participate in the study). We have followed the proband (patient V:1) for 8 years. Previous clinical data were available on patient IV:5 that were retrospectively analyzed. Clinical data of patients are summarized in a chronological order in Table 1. All affected members of the family were males who developed substantial myopia before school age. The refraction of patient IV:5 remained stable thereafter, while others experienced a gradual increase of myopic refraction throughout their life. Spherical equivalent of patients was between −5.0 and −21.0 diopters upon examination (patient IV:5 underwent corneal refractive surgery at the age of 40, therefore we used preoperative refractive data). The youngest patient had no complaints, while others suffered from photophobia, gradually

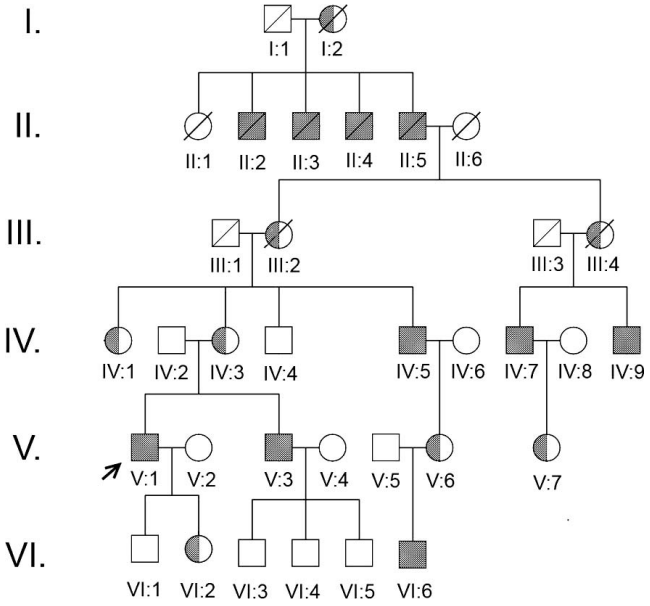


**Figure 1.** Pedigree of the family affected by X-linked high myopia. *Arrow* indicates the proband with L-opsin- LVAVA and M-opsin-MVAVA haplotype; *square*: male, *circle*: female, *diagonal lines* indicate deceased family members, *blackened symbol* indicates clinically affected individuals, and *half blackened symbol* indicates heterozygotes.

increasing difficulty identifying mixed colors and distinguishing between small color hue differences. Patients complained neither of night blindness nor a declined in their visual acuity. Even the oldest living affected family member (IV:5, 62-years old) considered his visual acuity satisfactory to maintain his regular work and everyday life. A significant decline in visual acuity could only be detected in the two oldest patients (IV:5, IV:7), both older than 50 years. A deutan/protan color blindness developed gradually and simultaneously along with the visual acuity decline in these patients, which could not be detected in younger individuals. However, patient VI:6 was found to have a mild deutan color vision defect with pseudoisochromatic charts and the Nagel anomaloscope at the age of 11 years. On the other hand, he performed well on the FM 100 hue test excluding a severe color vision defect. Ophthalmoscopic examination revealed myopic fundus changes with a central pigmented epithelial layer atrophy in the proband and patients V:3, IV:5 and IV:7, all older than 40 years. The 11-year-old patient (VI:6) had normal fundus appearance. Autofluorescent fundus images showed central patchy drop-outs in older patients (V:1, V:3, IV:5 and IV:7) indicating severe central pigmented epithelial layer atrophy. Central hyperreflective ring, a characteristic sign of cone dystrophies, was detected in patients IV:7 and VI:6. Spectral-domain OCT examination showed disruption of the pigmented epithelial layer, a thinning of the outer nuclear layer and photoreceptor outer segments in patients older than 40 years. (Fig. 2.)

### Electroretinography

The 11-year-old patient (VI:6) showed normal scotopic rod response and mildly decreased response upon maximal scotopic light stimulation (ERG 3.0). Moderately reduced amplitudes were registered under single photopic stimulation and a mild reduction was detected in the response for photopic 30-Hz flicker stimulation of cone photoreceptors. Patients V:1 (proband) and IV:7 showed severely reduced rod response, moderately reduced amplitudes to maximal scotopic light stimuli and undetectable electrical activity of cone

**Table 1.** Clinical Data of Affected Males

| ID | Age, y | Refraction | Best Corrected Visual Acuity | Color Vision | ERG | Fundus/OCT | Axial Length, mm |
|---|---|---|---|---|---|---|---|
| IV:5 | 40* | OD −4.25 D sph −0.5 D cyl<br>OS −5.25 D sph −1.5 D cyl | 20/20<br>20/20 | NA | NA | Normal fundus ODS | OD 25.16<br>OS 25.51 |
| | 57 | OD +1.75 DS<br>OS +1.0 DS | 20/25<br>20/40 | Deuteranomaly | NA | Mild PE degeneration ODS | NA |
| | 62 | OD +1.5 DS +1.5 DC<br>OS +1.0 DS +2.0 DC | 20/25<br>20/40 | Protan/deutan color blind | Missing cone response, reduced rod response | Moderate macular PE atrophy outer retinal thinning ODS | NA |
| IV:7 | 51 | OD −5.75 DS −2.0 DC<br>OS −6.5 DS −0.5 DC | 20/50<br>20/33 | Protan/deutan color blind | Mssing cone response, reduced rod response | Mild to moderate macular PE atrophy/outer retinal thinning ODS | OD 27.82<br>OS 27.57 |
| V:1 | 32 | NA | 20/20<br>20/20 | NA | NA | Normal macula with myopic periferal fundus | OD 31.44<br>OS 30.72 |
| | 38 | OD −20.0 D SE<br>OS −18.0 D SE | 20/20<br>20/20 | Normal color vision | NA | Mild macular PE atrophy with myopic periferal fundus | NA |
| | 46 | OD −21.0 DS −4.0 DC<br>OS −19.75 DS −2.5 DC | 20/20<br>20/25 | Protan/deutan defects† | Missing cone, reduced rod response | Moderate PE atrophy with myopic periferal fundus | OD 33.11<br>OS 32.28 |
| V:3 | 42 | OD −22.5 DS +2.0 DC<br>OS −24.5 DS +2.0 DC | 20/20<br>20/20 | Protan/deutan defects† | NA | Normal macula with Myopic periferal fundus / normal OCT | OD 31.35<br>OS 32.49 |
| VI:6 | 11 | OD −6.25 DS −3.75 DC<br>OS −6.5 DS −3.5 DC | 20/40‡<br>20/20 | Normal color vision | Reduced cone response, normal rod response | Normal fundus | NA |

Age represents the age at corresponding examinations. OD, right eye; OS, left eye; NA, not available.
* Patient IV:5 underwent corneal refractive surgery at the age of 40. Refraction values represent preoperative data.
† Normal with FM 100 Hue and Nagel anomaloscope, but shows both protan and deutan defects with pseudoisochromatic test plates.
‡ Patient VI:6 has an amblyopic right eye.

photoreceptors under photopic conditions in response to single or flicker stimuli. Patient IV:5 (62-years old) showed moderately reduced rod response, mildly reduced amplitudes to maximal scotopic light stimuli, and undetectable electrical activity of cone photoreceptors under photopic conditions in response to single or flicker stimuli (Fig. 3).

## Molecular Genetic Investigations

**Array CGH.** No chromosomal rearrangements or copy number variations of the X chromosome could be detected with array CGH (data not shown).

**RPGR ORF15 Analysis.** Mutations in the *RPGR* gene on Xp21 are the most common causes of X-linked cone dystrophy. ORF15 of *RPGR* gene has been identified as a mutation hotspot.[39,40] In the proband we found an already known in-frame 12-nt deletion polymorphism in nucleotides 3074_3085 resulting in elimination of four amino acids (rs201134185, c.3074_3085delTGGAAGGGGAGG, p.Val1025_Glu1028del). The deletion has no clinical significance.

**Clinical Exome Sequencing.** We detected 47 hemizygous variants in the proband and 50 heterozygous variants in the proband's mother on the X chromosome. Seventeen of these variants were found in both samples (Table 2). Ensembl, dbSNP, and Exac databases checked the minor allele frequencies of these variants. Filtering for minor allele frequencies (MAF) and clinical significance yielded three variants putatively associating to diseases in our pedigree. The c.1843G>T (p.Ala615Ser) variant in the *CACNA1F* gene had a MAF less than 0.01 and the gene is known to be associated with X-linked incomplete congenital stationary night blindness and Åland island eye disease.[41,42] The mutation could not be detected in any other family members by Sanger sequencing. We found two variants (c.532A>G, p.Ile178Val and c.538T>G, p.Ser180-Ala) in the *OPN1LW* gene and one variant (c.532A>G, p.Ile178Val) in the *OPN1MW* gene, which are known to form the LVAVA and MVAVA rare interchange haplotypes in the L- and M-opsin genes, respectively. No other nucleotides were detected in corresponding positions in any of the reads indicating only mutated opsin genes in the array. Sanger sequencing unequivocally confirmed these haplotypes with only one nucleotide detectable at each position in every affected family member. However, there were nucleotides coding a leucine and a methionine simultaneously detected at amino acid position 153, originating from the L- and M-opsin genes, as expected (Fig. 4). Based on these results we can conclude that the LVAVA and MVAVA haplotypes cosegregated with the disease in the entire family. We could not detect any additional nucleotide variations including SNPs or any other mutations in the L- and M-opsin genes of the proband and his mother. *OPN1MW2*, an additional M-opsin gene was only detected in the mother.

## Discussion

We have investigated a three-generation family with X-linked high myopia. Initially, the proband was suspected to suffer from nonsyndromic X-linked high myopia. However, diminished rod and extinguished cone response upon ERG examination indicated a simultaneous X-linked cone dystrophy confirmed by macular dystrophy and combined red and green color blindness (blue monochromatism) of older family members. We failed to detect any mutations in the ORF15 of the *RPGR* gene, a known locus for X-linked cone dystrophy.[39,40] X chromosome–specific high-resolution array CGH detected neither rearrangements nor copy number variations in the proband. Clinical exome sequencing analysis identified three putative variants including c.1843G>T (p.Ala615Ser) in the *CACNA1F* gene and the LVAVA and MVAVA rare interchange haplotypes in exon 3 of the L- and M-opsin genes, respectively. The pathogenic role of the *CACNAF1* mutation was already

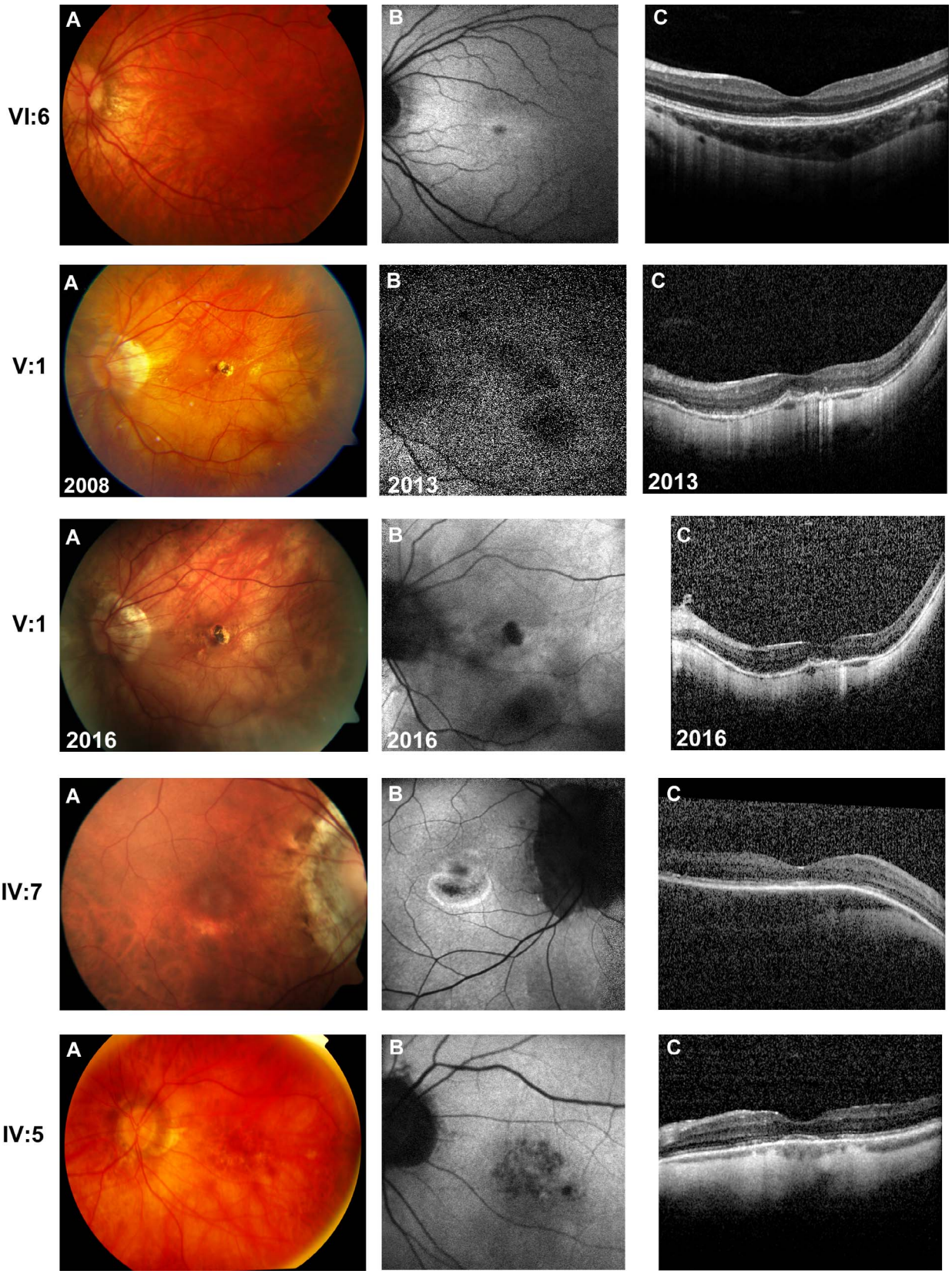


FIGURE 2. Findings of ophthalmic imaging examinations. Color fundus photographs (**A**) autofluorescent fundus photos (**B**) and SD-OCT images (**C**) for left eyes of three patients (VI:6, V:1, IV:5) and for right eye of one patient (IV:7). VI:6, aged 11 years, had normal fundus imaging, whereas all the other patients who were 38 years and older (V:1, IV:7, IV:5) showed myopic fundus changes and macular abnormalities with mild to moderate pigment epithelial atrophy. FAF images of VI:6, IV:7 patients showed central hyperreflective ring, a characteristic sign of cone dystrophies.

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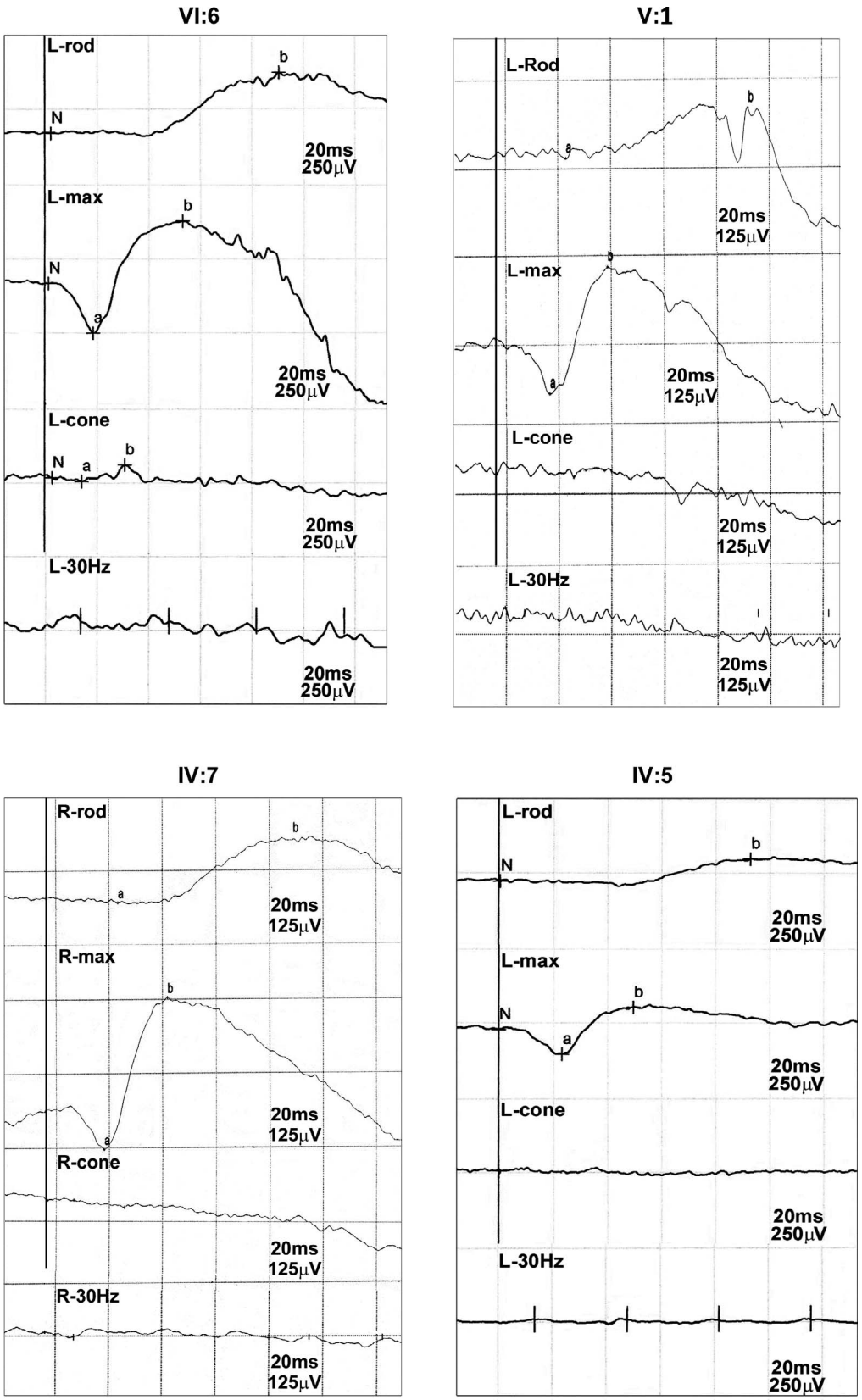


FIGURE 3. Electroretinography recordings of affected individuals. Dark-adapted 0.01 ERG (rod response, L-Rod, R-Rod); dark-adapted 3.0 ERG (maximal combined rod-cone response, L-max, R-max), light-adapted 3.0 ERG (single-flash photopic ERG, L-cone, R-cone), light-adapted 3.0 flicker ERG (30-Hz flicker, L-30 Hz, R-30 Hz). Electrorintography images related to patient age. The youngest patient showed (VI:6) normal rod and reduced cone response. Older patients (V:1, IV:7, IV:5) showed mildly reduced rod responses and moderate to severe cone dysfunction.

**Table 2.** Summary of Variations Detected Both in IV:3 and V:1 Family Members Using Clinical Exome Sequencing

| Gene | Coverage, -Fold IV:3 | Coverage, -Fold V:1 | SNP ID | Nucleotide Change | Amino Acid Change | MAF |
|---|---|---|---|---|---|---|
| *VCX* | 61 | 6 | rs75562767 | c.209C>G | p.Ala70Gly | 0.67 |
| | 104 | 15 | rs80291436 | c.311T>C | p.Leu104Pro | 0.46 |
| | 117 | 29 | rs78342118 | c.581C>T | p.Pro194Leu | 0.42 |
| *CACNA1F** | 31 | 12 | rs150417702 | c.1843G>T | p.Ala615Ser | <0.01 |
| *SLC16A2* | 45 | 15 | rs6647476 | c.97T>C | p.Ser33Pro | 0.66 |
| *KIAA2022*† | 143 | 57 | rs41306133 | c.2801A>G | p.Asn934Ser | 0.01 |
| *MAGT1* | 90 | 18 | rs145245774 | c.1028T>G | p.Val343Gly | 0.13 |
| *DIAPH2*‡ | 150 | 55 | rs20361 | c.1275C>A | p.Phe425Leu | <0.01 |
| *RBMXL3* | 29 | 13 | rs6643947 | c.3145A>G | p.Arg1049Gly | 0.11 |
| *LOC644717* | 25 | 17 | rs6528273 | c.1714G>T | p.Val572Phe | 0.66 |
| *SAGE1* | 66 | 23 | rs4829799 | c.2414T>C | p.Leu805Ser | 0.22 |
| *CSAG1* | 91 | 45 | rs2515848 | c.185A>G | p.Lys62Arg | 0.42 |
| *TMEM187* | 106 | 34 | rs2266890 | c.422C>T | p.Ser141Leu | 0.31 |
| | 111 | 34 | rs7350355 | c.445A>G | p.Met149Val | 0.37 |
| *OPN1LW*§ | 135 | 55 | rs145009674 | c.532A>G | p.Ile178Val | 0.02 |
| | 132 | 55 | rs949431 | c.538T>G | p.Ser180Ala | 0.2 |
| *OPN1MW*§ | 418 | 96 | rs375538821 | c.532A>G | p.Ile178Val | 0.02 |

* Associated phenotype: Aland Island eye disease, X- linked cone-rod dystrophy, X-linked congenital stationary night blindness.
† X-linked mental retardation.
‡ Premature ovarian failure.
§ Red-green color vision defects, BED, X- linked cone dystrophy, Blue cone monochromatism, X- linked nonsyndromic high myopia.

questioned by the noncorresponding clinical phenotype. Moreover, the mutation could not be detected with Sanger sequencing in any other family members, ultimately excluding it as a cause of the disease. On the other hand, Sanger sequencing in all affected family members, making them the only possible cause of the disease, could confirm the LVAVA and MVAVA haplotypes. Normal haplotypes were detected with neither of the sequencing methods indicating a mutated-only genotype in the opsin gene array in all patients. The LVAVA haplotype was found to cause the disease in the first two families reported with BED and also in some other families reported thereafter with the same genetic background and comparable phenotype.[14,27,29,32] However, findings in our family show striking differences when compared with these reports: our patients retained normal visual acuity and color vision even in their middle ages and only thereafter did the disease lead to progressive blue monochromatism and macular dystrophy, a phenotype previously not described in association with rare interchange haplotypes. It was previously shown, that rare interchange haplotypes cause skipping of exon 3 introducing a frameshift and a premature stop codon.[29,35] However, in contrast to the LIAVA haplotype, both MVAVA and LVAVA haplotypes were shown to produce some amount of full length mRNA.[29] Accordingly, direct opsin inactivation by the LIAVA haplotype leads to congenital color vision defect, while LVAVA does not lead the direct color vision defect on its own. Color vision defect observed in LVAVA patients was rather to be accounted for by the absence of either the L- or the M-opsin gene within the first two positions of the L/M-opsin gene array.[26,27,43] This observation explains the normal color vision in our young patients and also the nonsyndromatous high myopia associating to the LVAVA haplotype in two Chinese

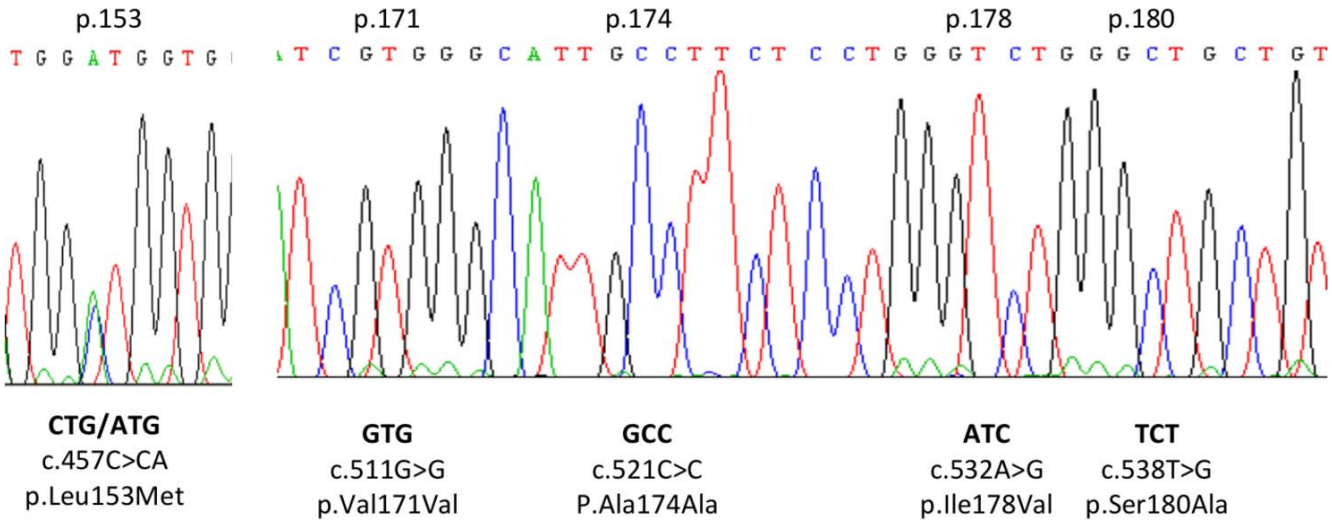


**Figure 4.** Electropherogram showing opsin variants detected by Sanger sequencing in the proband.

families.[33,34] The LVAVA haplotype differs from other interchange haplotypes in another aspect as well. BED was defined as a nonprogressive eye disease and it was first described in families with the LVAVA haplotype. In contrast to that, SD-OCT and adaptive optics scanning light ophthalmoscopy analysis showed disruption of the cone photoreceptor mosaic and degenerative changes in the macula of patients with only LVAVA haplotype indicating a progressive macular degeneration.[27,43] In contrast to that, LVAVA haplotype associated to nonsyndromic high myopia in two Chinese families.[33,34] However, the oldest family member (75-years old) having only light perception visual acuity was excluded from further clinical investigations due to senile cataract making any ophthalmologic examinations impossible in the first family. The next oldest patient was only 37-years old. Besides, there was a patient with mild protanomaly reported from the same family,[33] and affected male family members had a visual acuity between 0.1 and 0.2 in the second family.[34] It is also to be noted, that three patients in this family displayed mild to moderate reduction in cone response upon ERG examination. Our results indicate that late-onset progressive cone dystrophy might be a possible outcome in seemingly nonsyndromic high myopia families with the LVAVA and MVAVA exon 3 interchange haplotype in the opsin genes. Accordingly, subtle color vision defects and ERG alterations detected in these Chinese families might indicate future clinical symptoms. Normal color vision detected in young affected individuals in the present family indicates that both haplotypes result in a certain amount of normally spliced opsin mRNA and functional opsin proteins. A progressive macular dystrophy could be demonstrated in patients older than 40 years, which finally led to combined red and green color blindness in the two oldest patients. These data indicate that color vision defect only develops along with cone dystrophy as a consequence of the toxic effect rather than a direct opsin inactivation of the LVAVA/MVAVA haplotypes. Here, we confirm clinically for the first time that LVAVA and MVAVA haplotypes do not cause color vision defect or impaired vision in young individuals but cause late-onset progressive cone dystrophy leading to a decline in visual acuity and color vision defect in patients older than 40 years. This represents a novel phenotype that has not been previously reported to associate to any of the rare exon 3 interchange haplotypes. Our results add substantial new knowledge to the understanding of genotype–phenotype correlation and disease course of the corresponding rare exon 3 interchange haplotypes. Our report highlights that clinical phenotype associating to LVAVA/MVAVA exon 3 interchange haplotype in the opsin genes may only manifest in older individuals. However, further studies are needed to elucidate the course of the disease in individuals older than here presented. Moreover, recognizing this novel genotype–phenotype association could be essential as soon as gene therapy is applied in opsin-related diseases. In that case, this genotype could be of special interest with its long asymptomatic period providing a large therapeutic time window.

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